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Ophthalmic inserts prepared either by extrusion or by freeze-drying: technological and biopharmaceutical characterization

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To all the people who have always believed in me

“Enthusiasm is one of the most powerful engines of success. When you do a thing, do it with all your might. Put your whole soul into it. Stamp it with your own personality. Be active, be energetic, be enthusiastic and faithful, and you will accomplish your object. Nothing great was ever achieved without enthusiasm.”

Ralph Waldo Emerson

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Abstract

The main goal of this thesis is the development of an ophthalmic biodegradable insert containing fluocinolone acetonide (FA), to apply in the posterior segment of the eye. The inserts under study were prepared using the hot-melt extrusion technique (HME). In a previous study, an insert based on hydroxypropyl cellulose (HPC-FA) was selected as the best with respect to a wide range of devices tested. New inserts containing amylo-maize starch N-400 (AMYLO), corn starch (CS) or corn starch with magnesium stearate (CS-MS) were prepared. All inserts were added of 3% FA (AMYLO-FA, CS-FA and CS-MS-FA, respectively). Swelling and dissolution properties of the new inserts were analysed in isotonic buffer saline (PBS) by a visual examination. During the examination time (up to 12 days), AMYLO-FA produced no changes in terms of dissolution and gel-like transformation in addition to an initial hydration and light swelling compared to the inserts containing CS; so it has appeared the most suitable for further studies.

The physicochemical characteristics of the selected inserts (HPC-FA and AMYLO-FA) were determined by a) in *vitro* drug release and by b) differential scanning calorimetry (DSC). Drug release performance of AMYLO-FA and HPC-FA inserts was comparatively evaluated using Gummer-type diffusion cells up to 52h, maintaining the sink conditions (continuous receiving phase replacement), to evaluate the device influence on the FA release independently to the surrounding environment. During the time observed, HPC-FA released the drug too quickly (89% of drug released) for our goal unlike AMYLO-FA that showed a remarkable reduction of FA release (36.13%). The test on AMYLO-FA was continued for 8 days and a slow but continuous release was obtained to reach 63.66% of FA at the end of the experiment. A controlled drug delivery with zero order kinetics could be hypothesized when AMYLO-FA release process reached the steady-state. The release rate of FA from AMYLO-FA insert was also determined when the receiving phase (PBS) was totally replaced every 24h and 48h simulating the biological conditions: vitreous humor turnover is considered stagnant. For both performed experiment protocols FA release from AMYLO-FA insert was linear during the 25-day sampling period but the release rate decreased by half for sampling 48 hours. Then it would seem that the release of the drug from the device was mainly influenced and driven by the receiving environment rather than from the insert.

The DSC analysis of AMYLO-FA (as physical mixture and extruded) suggested that no polymer-drug interaction occurred using the HME and this means that the release of the drug might depend only on the modified polymer structure.

The second part of this thesis aimed at tuning up cylindrical matrices by freeze-drying for administration of vancomycin (VA) in the precorneal area to produce a sustained release of drug and consequently to reduce the number of applications. The matrices prepared contained hydroxypropyl methylcellulose (HPMC) and VA in ratio 0.25 to 1 and were subjected to a) in *vitro* release study and b) DSC analysis. HPMC-VA matrix released the drug very slowly for our goal, may be due to a drug-polymer interaction during the freeze-drying process. DSC analysis of lyophilized matrix and the single components would suggest this hypothesis.

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I. Introduction

1. The Anatomy and Physiology of the Eye

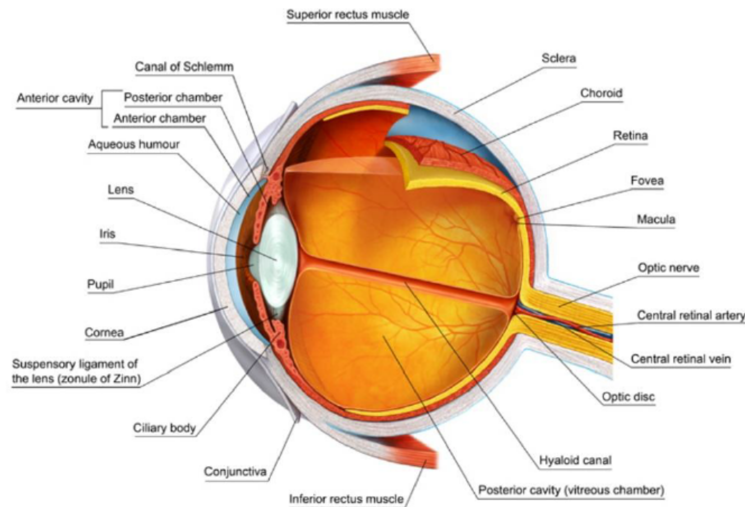


Figure 1 - The anatomy of the eye.

The eye is an isolated sensory organ and is located in the front upper part of the skull. The outer tissues of the eye consist in three layers. The outermost layer consists of the cornea and sclera (fibrous tunica), which provide protection for internal structures. The middle coat, the *uveal tract*, is a vascular layer, which has a nutritive function and is composed by choroid, ciliary body and the iris. The innermost layer is the retina (*nervous tunica*) containing photoreceptors and is involved in the reception of visual stimuli. The inner eye is divided by the lens, which separates the aqueous and vitreous humours; instead, the iris separates the aqueous humor into the anterior and posterior chambers. A brief description of the eye structures is shown below starting from the outside:

a. The Precorneal Tear Film. The precorneal tear film is a very thin layer, which is continuously wetting the corneal epithelium, the conjunctiva and the walls of the conjunctival cul-de-sac. The tear film has many functions, i.e., provides a smooth uniform refractive surface, lubricates the ocular surface, protects against bacterial infection, and removes cellular debris and foreign matter. This moisturizing action is important not only for the maintenance of a clear cornea but also for the eyelids movement.

The tear film is composed by three layers: lipid, aqueous, mucin coats. The outermost layer is the lipid layer which is approximately 0.1 μm thick and is composed by wax and cholesterol; its function is to reduce the evaporation from the underlying aqueous phase and, in this way, preventing the cornea from drying out. The aqueous

layer lies below the lipid layer and is the larger component of the tear film (6-10 μm thick), consisting of watery lacrimal secretion provided by the numerous accessory lacrimal glands, most of which are situated in the upper conjunctival fornix. The innermost layer is the mucoin coat, which is secreted by conjunctival globet cells and glands of Manz. Mucin is involved in adhesion of the aqueous phase to the underlying corneal epithelium and acts as a wetting agent reducing the interfacial tension between corneal epithelium and tears (Saettone et al, 1999).

The water-soluble elements of the tears are electrolytes, proteins and peptides such as albumin, lysozyme, lactoferrin, cytokines, growth factors etc (Lemp, 2008).

The tear film in the eye is constantly replenished and this process is connected to the blinking, which sweeps the excess fluid by the nasolacrimal duct. The lacrimal fluid in humans has a volume of 7 μL with pH 7.4 (Shahwal, 2011).

b. The Cornea. The cornea is an avascular, transparent structure composed of five layers with a total thickness of 300 – 500 μm. These layers include: epithelium, Bowman's membrane, stroma, Descement's membrane, and endothelium.

The epithelium plays a critical role in the maintenance of a barrier against tearborne agents and in a balanced stromal hydration. It is composed of five to seven cell layers, with a total thickness of 35-50 μm. The cells at the base are columnar but, as they are squeezed forwards by new cells, they become flatter identifying three groups of cells: basal cells, an intermediate zone of 2-3 layers of polygonal cells (wing shaped) and squamous cells. The permeability of the intact corneal epithelium is low, suggesting that tight junctions exist between the cells of the outer layer. The outer layer of the surface cells possess microvilli on their anterior surface may be to anchor the precorneal tear film. The cells of the basal layer show extensive lateral interdigitation of plasma membranes and are, therefore, relatively permeable. Immediately adjacent to the epithelium there is a less ordered region of the stroma, the Bowman's membrane followed by stroma, a hydrated (75-78% water) matrix of collagen fibrils and glycosaminoglycans (GAGs), which constitutes 90% of the corneal thickness. As structure purely hydrophilic, the stroma can act as barrier for very lipophilic substances. This arrangement is followed by Descement's membrane and corneal endothelium. Descement's membrane forms the basement membrane for the corneal endothelium. Corneal endothelium is a monolayer of polygonal cells, most of which are hexagonal in shape with about 20 μm diameter and 4-6 μm thickness. These cells play a key role in maintaining corneal transparency through their

transport, synthetic, and secretory functions (Sunkara and Kompella, 2003). The endothelium is in contact with aqueous humor of anterior chamber, and it is crossed by a passive flux of water towards the stroma while an active pump mechanism generates a flux in the opposite direction controlling corneal turgescence.

c. The Conjunctiva. The conjunctiva is a thin, transparent mucous membrane lining the inside of the eyelids and is continuous with cornea. The conjunctiva can be divided into three layers: (a) an outer epithelium, a permeability barrier, (b) substantia propria, containing nerves, lymphatics, and blood vessels, and (c) submucosa, which provides a loose attachment to the underlying sclera (Sunkara and Kompella, 2003). The conjunctiva, lining the lids, is vascular (palpebral conjunctiva) while on the globe is transparent (bulbar conjunctiva). The area between the lids and the globe is named conjuctival sac.

d. The Sclera. Sclera is the white or opaque fribrous tissue that forms the external protective coat. It is composed, mainly, of collagen fibers, which have a haphazard arrangement that causes the opaque scleral effect (Boddu et al., 2013). Other elements present in the sclera are proteoglycans, elastin, proteins and cellular components and water. The main function of this tissue is to provide a protective layer for the internal components of the eye.

e. The Anterior Chamber and Aqueous Humor. The anterior chamber varies in depth and is bordered by cornea in the front and the pupil and iris diaphragm in the back. It is filled with aqueous humor produced by the ciliary epithelium in the posterior chamber. Aqueous humor is formed from blood plasma by mechanism of diffusion, ultrafiltration and active transport. The ciliary processes of the human eye produce aqueous humor at a rate of 2-3 μL/min. Before the aqueous humor reachs the anterior chamber, it flows throught the posterior chamber (Boddu et al., 2013). Furthermore, the aqueous humor production and the intraocular pressure are maintained by membrane transport processes (Sunkara and Kompella, 2003).

f. The Iris – Ciliary Body. The iris, ciliary body and choroid comprise the vascular uveal coat of the eye. The anterior iris is immersed in the aqueous humor, which enters the iris stroma through openings or crypts along its anterior surface. The iris receives its blood supply from the major arterial circle, which lies in the stroma of the ciliary body near the iris root. The ciliary body can be divided into the following regions: nonpigmented ciliary epithelium, pigmented ciliary epithelium, stroma, and

ciliary muscle. The main arterial blood supply to the ciliary body is through the long posterior and the anterior ciliary arteries (these capillaries are fenestrated and leaky) (Sunkara and Kompella, 2003).

g. The Lens - The lens is a transparent tissue, consisted of 65% water and the rest mainly by proteins. Anteriorly, the lens is in contact with the pupillary portion of the iris, and posteriorly it fits into a hollow depression of the anterior vitreous surface. The major components of the lens are capsule, epithelium, and lens fiber cells. The lens capsule is acellular, transparent, elastic, and acts as an unusually thick basement membrane that encloses the epithelium and lens fiber cells. Membrane transport proteins in the lens play an important role in cell volume regulation, nutrient supply, and lens transparency. All cells of the lens are interconnected by gap junctions (Sunkara and Kompella, 2003).

h. The Retina. The retina is mainly divided in two layers: neural retina (inner layer) and retinal pigment epithelium (RPE - outer layer). The neural retina comprises rods, cones, bipolar cells and ganglionic cells, which are involved in the conversion system of the light into electrical impulses that provides for the normal vision. RPE is a non-visual portion located between the neural section of the retina and choroid and consists of a layer of melanin–containing epithelial cells. Melanin helps in absorption of stray light rays entering the eyeball and prevents the reflection of scattering of light. The RPE forms a blood-retinal barrier through tight junctions that enable the neighboring cells to connect each other. These cells regulate the trans-epithelial transport of various molecules through apical tight junctions, which retard the diffusion through the paracellular spaces. The numerous microvilli on the neural retinal section help the absorption of various nutrients and thereby maintain the viability of the neural retina. RPE expresses certain efflux pumps, which block the entry of xenobiotics from the extravascular space of the retina (Boddu et al., 2013).

i. Vitreous humor. The vitreous humor consists in a viscous gel-like structure and represents the 80% of the total volume of the eye. This fluid fills up the space between the lens and the retina. Vitreous humor is composed by water (99%), hyaluronic acid, hyalocytes, inorganic salts, sugar, ascorbic acid and a network of collagen fibris. The network of non-branching collagen fibers with hyaluronic acid imparts viscosity to the vitreous humor (Boddu et al., 2013). While the aqueous humor is continuously replenished, vitreous humor has a lower turn over. It serves as a mechanical buffer for the surrounding tissues, exerts an important role in the

transmission of the light to the retina, and is involved in the maintenance of the intraocular pressure (Gajraj, 2012).

2.The Ophthalmic Therapy

Topical therapy is the most commonly used for treatment of diseases of the anterior segment of the eye such as ocular surface diseases (conjunctivitis, dry eye, keratitis, etc.), glaucoma and anterior uveitis. These dosage forms like eye drops and suspensions are convenient and easy to instil but suffer from inherent drawbacks that lead to an ocular low bioavailability of drug due to, for example, the tear turnover, poor corneal permeability, nasolacrimal drainage, systemic absorption connected to side effects. To overcoming some of these problems and obtain an effective therapy, we can consider drug delivery systems, with a sustained drug release, both to prolong therapeutic activity and interact better with the ocular barriers or to reach specifically the drug's site of action.
However, topical therapies are limited for treating disorders of posterior segment due to the greater diffusional distance as well as anatomical and physiological barriers in the eye. In this case intravitreal drug injections have been explored for delivering drugs to target tissues in the eye at therapeutic concentrations.

2.1 The Topical Ocular Administration

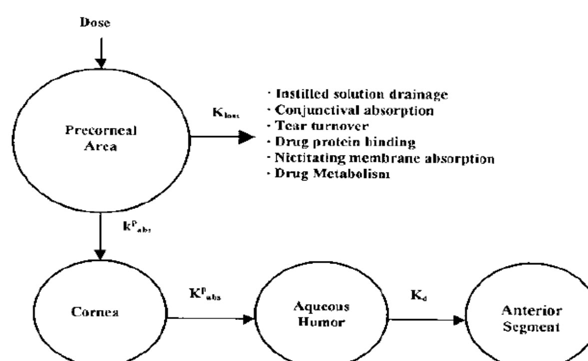


Figure 2 - Model Showing precorneal and intraocular events following topical ocular administration of a drug.

The formulation intended to administer to the eye surface may be required either to act at the precorneal area or to penetrate through the ocular structures to act into the interior of the eyeball. First of all there are some anatomical and physiological constrains to take into account when we use a topical administration such as cul-de-sac limited volume, the solution drainage, lacrimation, tear turnover, drug spillage for blinking, conjunctival-scleral absorption, enzymatic metabolism, and protein binding.

The ocular bioavailability remarkably depends on a suitable contact time with the cornea. Instilled solution drainage away from the precorneal area (within 5 minutes after instillation in humans) has been shown to be the most significant factor reducing the permanence of drug in this area. The natural tendency of the cul-de-sac is to reduce its fluid volume to 7-10 μL because of the blinking. A typical ophthalmic dropper delivers 30 μL, but due to the aforementioned reason, most of the active is rapidly lost through nasolacrimal drainage immediately after the following administration. This drainage mechanism may then cause the drug to be systemically absorbed across the nasal mucosa or the gastrointestinal tract leading to important side effects. The tear turnover acts to remove drug solution from the conjunctival cul-de-sac. Normal human tear turnover is approximately 16% per minute. Moreover, absorbed drug may set off the eye through the canal of Schlemm or via absorption through the ciliary body of suprachoroid into the episcleral space. In addition, even if the drop's proteinaceus content (albumin, globulins, lysozyme) is very low (about 0.7%), the continuous tear turnover produces new proteins most of which can bind the drug molecules. Enzymatic metabolism may contribute for further loss, which can occur in the precorneal space and/or in the cornea.

The lacrimation (tear production), wihich leads to a diluition of the pharmaceutical form, also influences the ocular drug absorption. It can be induced by many factors such as the type and amount of drug, pH and tonicity of the dosage form, use of adjuvants.

All these factors may influence the ocular drug bioavailability that has been estimated to be 1-2% (or less) of the instilled dose (Macha et al., 2003).

Another important factor is the absorption of the drug into the conjunctiva and sclera, the so-called conjunctival/scleral pathway. Drug loss through this route of absorption may be significant. Conjunctival-scleral pathway is fairly permeable to hydrophilic and large molecule and may serve as a route of absortion for some larger molecules such as proteins and peptides (Urtti, 2006).

The mechanism of permeation of the drug through ocular tissues to arrive into internal structure of the eye is the passive diffusion governed by the Fick's fist law shown above:

$$J = -D \frac{dC}{dx}$$

where **J** is the flux rate through the membrane; **D** is the diffusion coefficient that depends on physico-chemical properties of the drug such as molecular size; **dC/dx** is the concentration gradient, i.e., the ratio between the difference of concentration to the two sides of the barrier and the thickness of the barrier. Another parameter that influences the flux of the drug through the ocular tissue is the partition coefficient (**K**), which indicates the affinity of the drug for the lipophilic or hydrophilic compartments. Equilibrium between the two characteristics (lipophilic or hydrophilic) is essential to promote the transocular permeation. It is possible to calculate partition coefficient value determining octanol/water partition coefficient.

If the topically administered drug must reach the anterior chamber of the eye, it has to pass through the corneal layers. Corneal epithelium, the first barrier, can be crossed though two pathways: transcellular (through the cells) and paracellular (between the cells) pathway (Fig. 3). Lipophilic molecules and molecules with specialized transport processes prefer the transcellular route, whereas hydrophilic molecules lacking membrane transport processes prefer the paracellular route (Shahwal et al, 2011).

The mechanism of **transcellular** transport includes simple diffusion, facilitated diffusion, active transport, and endocytosis. For the most of the available drug molecules, no such transporters or receptors exist, and these drugs are transported by passive diffusion through the apical membrane and across the basolateral membrane to move across the cell. During the transcellular movement, the solute must interact with some components of the cell membrane. Physiochemical properties of the drugs that may influence drug penetration via the transcellular pathway, purely lipophilic, include: (1) the lipophilicity of the drug, reflected by its octanol-water partition coefficient; (2) its pKa (dissociation constant), which determines the amount of absorbed drug depending on its ionized or unionized form at a given pH; (3) molecular size.

Transport through the **paracellular** pathway is passive and only limited by the size and charge of the intercellular spaces. It is an aqueous route involving the diffusion of the solute between adjacent epithelial cells/endothelial cells restricted by the presence of the tight junctions or *zonula occludens* (ZO) (Sunkara and Kompella, 2003).

For most drugs, the multicellular layered corneal epithelium presents the greatest barrier to penetration while stroma and endothelium offer little resistance. Indeed, for

too much lipophilic drugs, the corneal stroma represents a rate-limiting barrier to access the inner eye and in this case it may act as a reservoir from which the drug will be slowly delivered to the aqueous humor. Finally, the moderate lipophilic corneal endothelium does not offer significant resistance to drug absorption (Ye et al., 2013). In fact, the drug should be both oil and water soluble to across the corneal epithelium (lipid barrier) then the aqueous humor (Shahwal et al, 2011).

In addition to the classical corneal pathway, there is a competing and parallel route of absorption via the conjunctiva and sclera, the so-called conjunctival/scleral pathway; comparing the corneal route with this absorption pathway, it results that the second route is a minor absorption pathway, even tough for some compounds its contribution is rather significant.

Ahmed and Patton (1985, 1987) investigated corneal versus noncorneal penetration of topically applied drugs in the eye. They demonstrated that noncorneal absorption could contribute significantly to intraocular penetration. Drugs can bypass the anterior chamber and distribute directly to the uvea and vitreous. For low corneal affinity drugs such as inulin, this absorption route may be particularly important. In another study, Ahmed et al. (1987) evaluated in *vitro* the barrier properties of the conjunctiva, sclera, and cornea studying the diffusion characteristics of various β-blockers. Permeability through the sclera of these compounds was higher than through the cornea related to their physicochemical properties. The results suggested that the pathway of absorption might be in part influenced by lipophilicity and that hydrophilic compounds seem to prefer the conjunctival/scleral route. Schoenwald et al. (1997) verified that a compound with a remarkable lipophilicity as rhodamine B did not pass through conjunctival/scleral barrier.

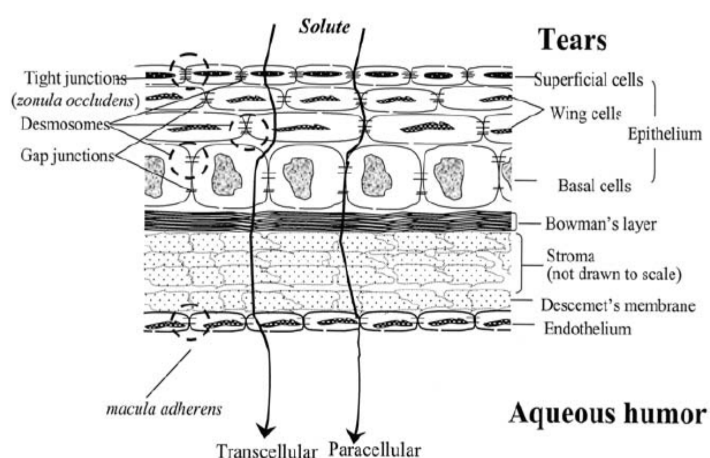


Figure 3 - The cornea. Cellular organizayion of various transport limiting layers.

2.2 Intravitreal, Periocular and Systemic Administrations

a. Intravitreal Administration. Intravitreal injection is the most direct approach for delivering drug to the vitreous humor and retina; however, this method of administration has been associated with serious side effects, such as endophthalmitis, cataracts, hemorrhage, and retinal detachment. In addition, multiple injections are usually required, further increasing the risk. Nevertheless, intravitreal injection continues to be the mode of choice for treatment of acute intraocular therapy.

The kinetic behaviour of intravitreally delivered drugs is influenced by the normal vitreous physiology. Mechanisms that may influence movements of molecules within the vitreous include diffusion, hydrostatic pressure, osmotic pressure, convective flow, and active transport. For small and moderately sized molecule diffusion is the predominant mechanism of transvitreal movement; for this kinds of molecules, the diffusion within the vitreous is generally unimpeded and similar to that observed in water or saline (Chastain, 2003).

b. Periocular Administration. Drugs usually do not reach therapeutically relevant levels of the active agent in the posterior segment via topical route. If significant concentrations are achieved at the back of the eye, they are usually the results of the redistribution from the systemic circulation, not local delivery. Consequently, to treat diseases of the posterior segment, drug must typically be administered intravitreally, periocularly, or systemically. Periocular drug administration, using subconjunctival, sub-Tenon's, or retrobulbar injection, is another and, in many cases, preferred, route for delivering drugs to the posterior segment.

Subconjunctival injection offers the advantage of local drug delivery without the invasiveness of intravitreal injection. This route also allows for the use of drug depots to prolong the duration of drug therapy and avoids much of the toxicity encountered with systemic administration. Drug concentrations in the eye are typically substantially higher following subconjunctival versus systemic administration, while systemic exposure is greatly reduced with subconjunctival dosing.

Maurice and Mishima (1984) point to direct penetration to deeper tissues as the main pathway of entry into the anterior chamber. The drug must saturate the sclera to enable the absorption by this route. This condition is followed by diffusion by various possible routes: laterally into the corneal stroma and across the endothelium, across trabecular mesh-work, through the iris stroma and across its anterior surface,

into the ciliary body stroma and into newly generated aqueous humor, and into the vitreous body via the pars plana and across its anterior hyaloid membrane. In addition to these pathways, depending on the injection volume, regurgitation out the dose site with subsequent spillage onto the cornea can lead to direct transcorneal absorption.

Sub-Tenon's injection involves delivery of drug, usually as a depot, between the sub-Tenon's capsule and sclera or episclera. This route of administration has the advantage of placing drug in very closely proximity to the sclera. Drug can subsequently diffuse through the sclera, which is quite permeable to a wide range of molecular weight compounds (Chastain, 2003).

c. Systemic Administration. This kind of administration is generally not preferred because of the several side effects. However, for drug delivery to the posterior segment or vitreous body, systemic administration could be the best choice depending to the drug's ability to penetrate the blood-retinal barrier or the blood-vitreous barrier and its systemic toxicity profile (Chastain, 2003).

3. Ocular Drug Delivery Systems

The pharmaceutical forms more commonly used in ophthalmic field can be classified on the basis of the physical form in:

- **Liquid** dosage forms: solutions and suspensions;
- **Semisolid** dosage forms: ophthalmic ointments and gels;
- **Solid** dosage forms: ocular inserts.

Although many techniques of instilling drugs to the eye have been experimented with, the use of eye drops remains the main method of administration for the topical ocular route. The two major physical forms of eye drops are aqueous solutions and suspensions. A homogeneous solution offers the potential of greater assurance, of uniformity of dosage and bioavailability and simplifies large-scale manufacture. Furthermore, the solution is more easily administrable with an excellent patient compliance even if it has, as fundamental drawback, the low bioavailability due mainly to the rapid and extensive precorneal loss, the high absorption via the conjunctiva and the nasolacrimal duct leading to systemic side effects. When the drug is not sufficiently soluble in hydrophilic vehicle a suspension can be formulated. A

suspension may also be desired to improve stability, bioavailability or efficacy. Most of ophthalmic suspensions are composed of the steroid antiiflammatory agents (such as prednisolone acetate, dexamethasone). The critical element of these formulations is the size of particles. Micronized particles (size <10 μm) are accepted in ophthalmic field to not cause irritation of sensitive ocular tissues and to help ensure that a uniform dosage is delivered to the eye. In any case there are a lot of constraints connected to preparation and administration of a stable suspension, i.e., they need to be adequately shaken before use to ensure the correct dosing and reach a moderate increase in bioavailability; and also the suspensions are expensive in terms of production costs.

To increase the ocular contact time and subsequently improve ocular bioavailability of the drug, high molecular weigh polymers can be added to the ophthalmic solutions and suspensions raising their viscosity. The most commonly used hydrophilic polymers in ocular field are polyvinyl alcohol (PVA) and polyvinyl pyrrolidone (PVP), and cellulose derivatives such as hydroxypropyl methylcellulose (HPMC), methylcellulose (MC) and polyacrylic acids (carbopols) (Shahwal et al., 2011).

There are many examples on the market of eyedrops containing rheological modifiers such as Fluaton®, containing PVA, Flarex® containing HEC, Flumetol® containing a mixture of PVA and HPMC, Retaine® HPMC™ lubricant eye drops containing HPMC.

There is a direct relationship between viscosity and bioavailability up to a plateau after which further increase in viscosity produces only slight or no increase in therapeutic effect. Many research works have demonstrated this correlation and led to the conclusion that ophthalmic liquid formulations should have viscosity in the range of 25 to 50 cP for a greatest permanence in the precorneal area. The addition of a 0.5% PVA solution to eye drops increases the viscosity to about 3 cP (water viscosity= 1 cP) and decreases the drainage rate of topically applied eye drops as compared with aqueous solution. The drainage rate decreases by a factor of 2-3 at viscosities up to 7.7 cP and further increase in viscosity from 7.7 to 75.8 cP is also associated with a decrease by a factor of 2-3. The rate of drainage decreases with additional PVA up to a maximum of 3%, after which there is no longer any advantage (Kebler et al., 1991).

Redkar et al. (2000) evaluated the influence of the viscosity of new possible commercial artificial tears on increasing the permanence time of the formulation in the precorneal area and on producing a stable tear film. MOISOL® eye drops (0.3% HPMC) was used as reference. As viscosity-imparting agents, HPMC and Dextran 70

were used individually or in combination. An aqueous formulation containing dextran 70 (0.1% w/v) and HPMC (0.7% w/v) appeared the best to maintain for a long time a stable tear film. The experimental formulation with a viscosity of 25.49 cP produced a longer permanence in the precorneal area of the eye drops (2h) than the reference MOISOL which has a viscosity of 42.30cP and a precorneal permanence time of 1,5 h. This seems to confirm that high viscosity is not necessarily correlated with a high bioavailability.

In addition to their thickening effect, many aforementioned polymers possess mucoadhesive properties, i.e. ability to establish adhesive, non covalent bonds with mucin layers coating corneal-conjunctival epithelium, giving ophthalmic drug delivery systems longer times of contact with the absorbing tissues. For an efficient mucoadhesive activity the polymers need to have one or more of the following features: strong hydrogen binding group, strong anionic charge, high molecular weight, sufficient chain flexibility, surface energy properties favoring spreading onto the mucus. The combination of mucoadhesion and viscosity is a target for an optimal activity (Saettone et al, 1999).

The principle semisolid dosage form used in ophthalmology is anhydrous ointment with a petrolatum base or a mixture of petrolatum and lanolin derivatives. Ophthalmic ointments containing antibiotics are used quite frequently following operative procedures. The reduction of dilution of medication via the tear film, resistance to nasolacrimal drainage, and increased precorneal contact time lead to a remarkable increase of bioavailability, whereas the blurred vision, and the discomfort by the patient are disadvantages that limit its use to a night treatment.

An aqueous semisolid gel base has been developed to provide significantly longer residence time into the cul de sac. The gel consists of a high molecular weight, crosslinked, polymer to provide high viscosity and optimum rheological properties for prolonged ocular retention. Only a relatively low concentration of polymer is required, so that the gel base is more than 95% water.

In-situ gelling systems are viscous polymer-based liquids that exhibit sol-to-gel phase transition on the ocular surface due to change of specific physic-chemical parameter (electrolyte composition, the change in temperature, pH). This kind of formulation has many advantages, i.e., an easy instillation, a prolonged residence time of the formulation on the surface of the eye (due to gelling), an easy, reproducible, accurate administration of a dose compared to the application of performed gels.

The ophthalmic inserts are solid devices designed to deliver the drug in the conjunctival cul-de-sac or in the posterior segment of the eye. The main objective of

ophthalmic inserts is to increase the contact time between the formulation and the conjunctival tissue to ensure a sustained release suited for topical or systemic treatment, resulting in an improved bioavailability.

3.1 Ophthalmic Inserts and Implants

Ocular inserts are defined as sterile preparations, with a thin, multilayered, drug-impregnated, solid or semisolid consistency devices placed into the cul-de-sac or conjunctival sac or introduced into the interior structure of the eye during surgery (implants), and whose size and shape are especially designed for ophthalmic application. Ocular inserts are sustained-release drug delivery systems (Lee et al., 2011). In general matrix implants are typically used to treat acute-onset diseases that require a loading dose followed by tapering doses of the drug during a 1-day to 6-month time period.

The main advantages to use ocular inserts are listed below:

- Increasing the contact time and thus improving bioavailability;
- Release of drugs at a slow, constant rate;
- Reduction of systemic absorption, lowering consequently side effects;
- Minor number of administrations and thus better patient compliance;
- Possibility of targeting internal ocular tissues through non-corneal (conjunctival scleral) routes.

One of the major disadvantages of ocular inserts resides in their ‘solidity’, which is felt by the patients (often oversensitive) as an extraneous body in the eye. This may constitute a difficult physical and psychological barrier for patient compliance. Furthermore, inserts can cause an interference with vision, causing some problems related to them placement (and removal, for insoluble types).

Depending on the type of polymeric material used to prepare the inserts, they can be classified in two main classes: a) nonbiodegradable and b) biodegradable ocular delivery systems.

a. Nonbiodegradable Ocular Delivery Systems. Ocular inserts composed by nonbiodegradable polymers are insoluble and therefore they have to be removed from the eye at the end of the short treatment.

The ophthalmic nonbiodegradable systems can be characterized as reservoir-type and implant-type. In the former type a drug core is slowly released across a nonbiodegradable semipermeable polymer or is released from a nonbiodegradable

polymer through the opening of a fixed area; in the latter type a nonbiodegradable free-floating pellet is injected intravitreally or a nonbiodegradable plug is anchored to the sclera.

Reservoir type is generally consisting of a combination of polyvinyl alcohol (PVA) and ethylene vinyl acetate (EVA). PVA, a permeable nonbiodegradable polymer, is used as the main structural element, and EVA, a nonbiodegradable polymer that is lipophilic and relatively impermeable to hydrophilic drugs, is used for the device's semi-permeable membrane. Indeed, the release of the drug from the reservoir-type system occurs by the diffusion of the water through the outer EVA coating, which partially dissolves the enclosed drug and forms a saturated drug solution that diffuses into the surrounding tissue depending on the concentration gradient. As long as the drug remains within the core, a near zero-order drug release kinetic with a relatively constant release rates is obtained when the steady state condition is achieved. The duration of drug release is mainly limited by the rate of drug dissolution within the reservoir.

On the other hand, the nonbiodegradable implants, devices to introduce in the internal structures of the eye, have a lot of drawbacks. For example large incisions and sutures or other form of anchoring can be necessary; the implantation and their removal can cause serious side effects such as retinal detachment, vitreous hemorrhage, and cataract formation.

Examples of nonbiodegradable devices reservoir-type to apply in the precorneal area available on the market are Mydriasert® and Ocusert®.

Mydriasert® is an oblong (4.3 mm x 2.3 mm), nonbiodegradable cylinder (reservoir) with rounded forms containing tropicamide and phenylephrine hydrochloride with ammonium methacrylate copolymer (Type A), polyacrylate dispersion (30%), and ethyl cellulose, as excipients. The reservoir is surrounded by a dyalisis membrane. When the system is introduced in the conjunctival sac, the tears enter into the device by osmotic effect and the active components are released through the membrane. It is mainly used to induce pre-operative mydriasis, allowing the mydriasis to be obtained quickly and to be maintained during surgery. It has to be removed from the conjunctival sac within two hours.

Figure 4 – Mydriasert®

Instead, Ocusert® (Fig. 5.a-b) is a nonbiodegradable conjunctival insert that provides sustained delivery (zero-order kinetics) of pilocarpine hydrochloride for the treatment of glaucoma. It consists of pilocarpine and alginic acid contained within a reservoir enclosed by two release-controlling membranes made of EVA copolymer surrounded by a ring to aid in positioning and placement (Fig. 5.a). While this product represented an innovation in ophthalmic drug delivery technology, its use was limited by complications associated with device insertion (Lee et al., 2011).

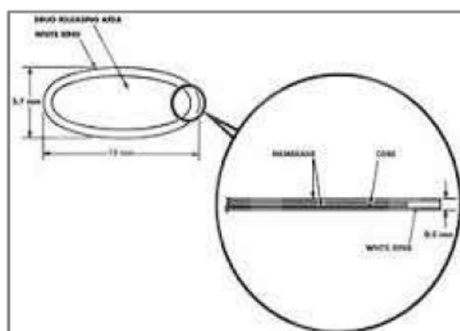


Figure 5.a - Ocusert® structure.

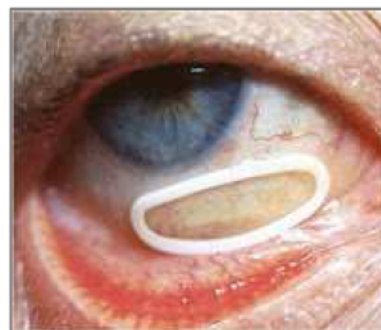


Figure 5.b - Ocusert ® placement in the eye.

Some examples of nonbiodegradable intravitreal implants are Retrisert® (Bausch and Lomb, Inc. Rochester, NY/pSivida Ltd.) and Iluvien® (formerly Medidur; Alimera Sciences, Inc., Alpharetta, GA, USA) containing fluocinolone acetonide (FA), Vitrasert® (Bausch and Lomb, Inc., Rochester, NY, USA) containing gancyclovir (GAC) and I-vation ™ (SurModics, Inc., Eden Prairie, MN) based on triamcinolone acetonide (TA).

Used for the treatment of chronic non-infectious uveitis affecting the posterior segment of the eye, Retrisert® is a disc-shaped intravitreal implant consisting of a matrix of fluocinolone acetonide coated with silicone and PVA attached to a 5.5-mm silicone suture tab. It is surgically inserted in the vitreous at the pars plana near the ciliary processes. The device has an initial drug delivery rate of 0.6 mg/day and reaches a steady-state delivery rate of 0.3–0.4 mg/day over roughly 30 months. Furthermore, Retrisert® received fast-track approval status and orphan drug designation from the U.S. Food and Drug Administration (FDA) for the treatment of chronic noninfectious uveitis of the posterior segment and diabetic macular edema (DME). The implant showed efficacy versus diabetic ocular edema, but was linked to the cataract onset and intraocular pressure increase after 3 years treatment (phase 3 clinical trial), indicating that, this implant is not suitable for long-term treatment (Lee et al., 2011).

Instead, the Iluvien™ intravitreal insert (Fig. 6), is a tiny cylindrical polyimide

tube, 3.5mm in length and 0.37mm in diameter, that contains 180 μg of FA. It has a reservoir fitted with end cap of PVA or silicone (in the 0.2mg/day dose version) that regulates the rate of drug release and provides nearly zero-order kinetics, with a slightly higher initial release rate that stabilizes over the long term. It is inserted using a proprietary 25-gauge injector system into the inferior vitreous to maximize drug exposure to the retina and minimize exposure to the anterior chamber. This implant exists in two different doses: 0.2 mg/day and 0.5 mg/day, which the lasting time is 24–30 months and 18–24 months respectively. By combining FA and a delivery device that provides for a unique long term, low dose delivery of FA to the back of the eye, it is believed that Iluvien™ has the potential to improve vision of those suffering from diabetic macular edema, while reducing common side effects of corticosteroids (Lee et al., 2011).

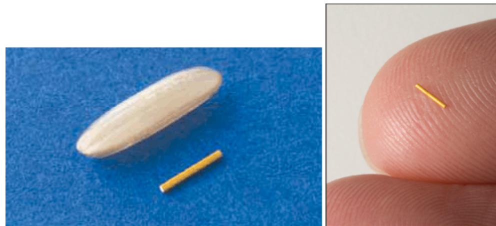


Figure 6 - Iluvien™: Comparison to grain of rice and on adult's finger.

Vitrasert® (Fig. 7) is a nonbiodegradable polymeric intravitreal ganciclovir implant developed for the treatment of cytomegalovirus retinitis. Ganciclovir (GAC) is encapsulated within a reservoir, encased by a PVA/EVA membrane, and diffuses out according to zero-order release kinetics when fluids enter the device and create a saturated solution. The device provides GAC sustained release for 5–8 months and achieves higher intraocular drug concentrations as compared with systemic administration. It is ideal for cytomegalovirus retinitis lesions that pose an immediate risk to vision (Lee et al., 2011).

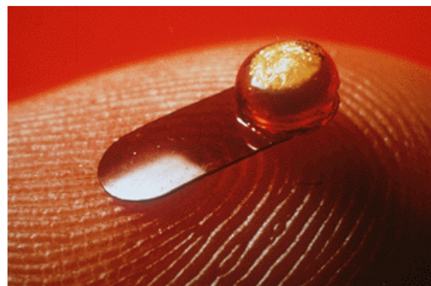


Figure 7 - Vitrasert®

I-vation^{TM} (Fig. 8) is a nonbiodegradable intravitreal implant consisting of a titanium helical coil coated with TA (925 µg) and the nonbiodegradable polymers poly(methyl methacrylate) and EVA. I-vation™ has a sharpened tip, used to do the incision for implantation, and its helical shape maximizes the surface area for drug coating and enables secure anchoring to the pars plana/sclera. It is predicted that this implant will have an in vivo sustained delivery of at least 2 years. I-vation^{TM} had been under investigation for the treatment of DME, but early trials evaluating the device reported the incidence of intraocular pressure elevation, conjunctival hemorrhage lenticular opacities, and endophthalmitis (Lee et al., 2011).

Figure 8 - I-vation^{TM}

b. Biodegradable Ocular Delivery Systems The peculiarity of biodegradable ODS is the presence of biodegradable polymers. Biodegradable polymer-based drug delivery systems show considerable promise for the treatment of ocular diseases and offer a potential solution to many of the limitations of conventional methods (systemic, oral and topical) for administration of ophthalmic drugs. Biodegradable polymer-based drug delivery can be used to achieve prolonged therapeutic drug concentrations in ocular target tissues with a controlled release rate.

The polymers used in biodegradable drug delivery systems have to be biocompatible and either leading to non-toxic metabolites or easily eliminated by endogenous metabolic pathways in the human body. An important characteristic for a biodegradable device is the mechanical strenght, i.e., it should be able to withstand to in vivo physical stress maintaining a structural integrity for a long enough period during the therapy. The drug release profile is depending on the choice of the polymer in particular to its biodegradation kinetics, physicochemical properties, and thermodynamic characteristics. The most used polymers for biodegradable inserts development are synthetic aliphatic polyesters of the poly-α-hydroxy acid family, which include poly (glycolic acid)(PGA), poly(lactic acid)(PLA), and the PGA/PLA copolymer poly(lactic-co-glycolic acid) (PLGA), poly(ε-caprolactone) (PCL), poly(glycolide-co-lactide-co-caprolactone) (PGLC), and others biomaterials such as

poly (ortho esters) (POE), polyanhydrides (PAH), polymethylidene malonate (PMM), polypropylene fumarate (PPF), and poly (N-vinyl pyrrolidone) (PVP). Among natural polymers, we can remember collagen, gelatin, chitosan, corn starch, amylo (high amylose starch). As shown in the Figure 9, the polymer chains gradually undergo a random scission that occurs more readly in center of the device. Once chains reach a threshold of reduced molecular weight, the shortened polymer chains become solubilized in the surrounding medium and are released from the device. This results in central hollowing of the implant with gradual mass loss of the implant.

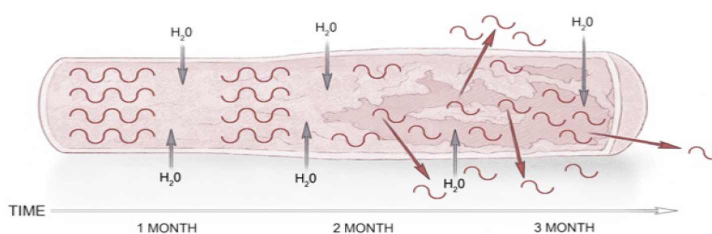


Figure 9 - Biodegradable insert erosion.

The Figure 10 illustrates what happens when a device undergoes to a bulk and a surface erosion process. In the former process (bulk erosion, that is shown in red) the dry implant shows porous structure prior the implantation and the drug molecules are interspersed in the pores and skeleton of the polymer (magnified view of Fig.10). After implantation, the implant is going towards a short burst phase of the drug (b-Fig. 10), during which the drug molecules diffusing out of the implant surface due to water penetration through the polymer pores present on its surface. Diffusion and random chain scission phase occurring immediately after burst phase. Consequently, the long polymer chains are split at random locations. At the same time, water molecules and drug molecules entering and exiting the implant from the core, respectively. The final phase consisting in the biodegradation and mass loss (d-f of Fig. 10) during which the polymer structure appears break down from internal cavitation and much of the drug molecules have already diffused out from the cavity. Structural changes and consequently implant shape alteration have occurred because of biodegradation process. Is clear that water molecules are still passing through the polymer and less drug is available for release. Towards the end of biodegradation, only the implant fragments remain.

The latter process (surface erosion, that is shown in blue) proceeds in the same way of the bulk process until burst phase (included). After this phase the surface erosion begins (i-Fig. 10), during which drug and polymer are both solubilized only on the surface of implant. The continuous surface erosion results in mass loss

from the surface of the device (j-l of Fig. 10). In this way, drug and polymer are released and solubilized from the surface of the implant, and its volume and surface are gradually reduced over time.

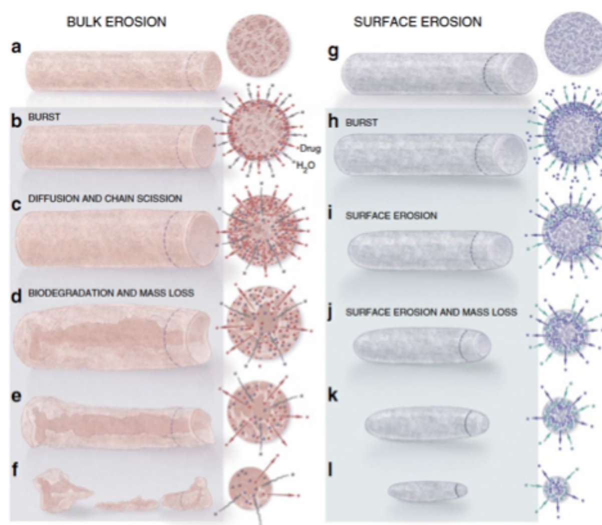


Figure 10 - Drug-release mechanisms and biodegradation of matrix implants.

An example of a biodegradable insert to applied in the precorneal area is Lacrisert®. It is a sterile, translucent, rod-shaped, water-soluble, biodegradable ophthalmic insert consisted of hydroxypropyl cellulose (HPC) to stabilize and thicken the precorneal tear film and prolong tear breakup time. It is indicated in moderate to severe dry eye syndrome. Lacrisert® side effects are typically mild and include blurred vision, ocular discomfort/irritation, eyelash matting/stickiness, photophobia, hypersensitivity, eyelid edema, and hyperemia (Lee et al., 2011).

Surodex (Allergan, Inc., Irvine, CA) is a rod-shaped biodegradable matrix (1.0 Å~ 0.5 mm) consisting of 60 μg of dexamethasone incorporated into the polymer matrix (poly(lactic-glycolic)-acid (PLGA) and HPMC. After insertion into the anterior chamber, it produces sustained and controlled release of dexamethasone over 7 days, achieving higher intraocular drug levels than conventional dexamethasone eye drops. It is used to control postoperative inflammation following cataract surgery.

Instead Ozurdex™ (formerly Posudex, Allergan Inc., Irvine, CA), is designed to the application into the posterior segment of the eye. It is a biodegradable implant consisting of 0.7 mg dexamethasone incorporated into a solid, rod-shaped PLGA copolymer (Novadur™, Allergan, Inc.) matrix. The implant is designed to release dexamethasone biphasically, with a peak doses for 2 months initially, followed by lower therapeutic doses for up to 6 months. Ozurdex received US FDA approval in June 2009 for the treatment of macular edema associated with retinal vein occlusion. During the clinical trials two main adverse events significantly and frequently occurred

in the treatment group: anterior chamber flare (5% for Ozudex vs. 0% for observation) and increased intraocular pressure (6% for Ozuderx and 0% for observation). Only 2% of total patients of the trial group using Ozurdex and only 1 patient (1%) in the observation group had an intraocular pressure increase of 10 mmHg or more from baseline at day 90 (Lee et al., 2011).

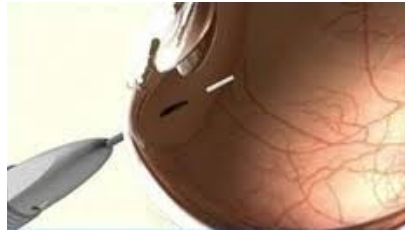


Figure 11 - Ozurdex™ implantation.

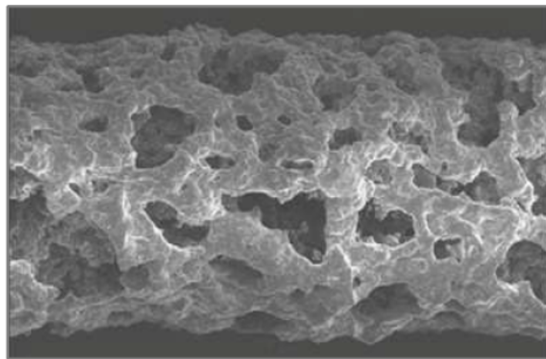


Figure 12 - Ozurdex™ after three weeks implantation in the target site.

II. Experimental Section

4. Aim

The main goal of this thesis is the development of an ophthalmic biodegradable insert containing fluocinolone acetonide (FA), to apply in the posterior segment of the eye. Furthermore, this device should easily circumvent constrains that usually affect the administration of drugs in the posterior segment of the eye (repeated injection, systemic absorption of the drug, uncontrolled drug release, drug precorneal loss, etc.).

The inserts under study were prepared by research group of Pharmaceutical Technology of the Department of Pharmaceutical Sciences - University of Milan (Prof. Gazzaniga) using the hot-melt extrusion technique. The matrices contained 3% FA and different polymeric materials.

In a previous study, insert made of hydroxypropyl cellulose (HPC-FA) was selected as the best with respect to a wide range of devices tested. Afterwards, new inserts containing amylo-maize starch N-400 (AMYLO), corn starch (CS) or corn starch with magnesium stearate (CS-MS) were prepared. All were added of FA (AMYLO-FA, CS-FA and CS-MS-FA, respectively). First of all, the behaviour of the new inserts in contact with isotonic buffer saline (PBS; 66.7mM, pH = 7.4) maintained at 37°C was analysed by visual examination: the swelling and the dissolution properties were monitored over time by using a digital microscope. On the basis of the results obtained by these experiments, AMYLO-FA appeared the most suitable to our goals.

The physicochemical characteristics of the inserts above selected (HPC-FA and AMYLO-FA) were determined by a) in *vitro* drug release analysis to determine the influence of the insert composition and the production technique on the profile of drug release and by b) differential scanning calorimetry (DSC) focused on the potential drug–polymer interaction and the structural changes that may occur during the hot-melt extrusion process.

The second part of this thesis aimed at tuning up cylindrical matrices by freeze-drying for administration of vancomycin (VA) in the precorneal area to produce a sustained release of drug and consequently to reduce the number of applications.

The matrices were subjected to the same tests above described (in *vitro* VA release analysis and DSC) to determine their physico-chemical properties.

5. Materials and Methods

5.1 Materials

The following products were used:

- ❖ High amylose maize starch (AMYLO) (Amylo-maize starch N-400) (Roquette S.p.a, Italy)
- ❖ Corn starch (CS), (Sigma Aldrich, Chemie GmbH, Germany)
- ❖ Magnesium stearate (MS), (Sigma Aldrich, Chemie GmbH, Germany)
- ❖ Hydroxypropyl cellulose (HPC, KLUCEL®HF- PM 1150000)
- ❖ Hydroxypropyl methylcellulose (HPMC, Methocel K4M premium EP, Colorcon, UK)
- ❖ Fluocinolone Acetonide (FA), (Sigma Aldrich, Chemie GmbH, Germany)
- ❖ Vancomycin Hydrochloride (VA) – European Pharmacopoeia (Sigma Aldrich, Chemie GmbH, Germany)
- ❖ All other chemicals and solvents were of analytical grade.

5.1.1 AMYLO

AMYLO is a polymer composed by native starches that represent the carbohydrate fraction of the grain or tuber, obtained after the separation of the other components via a wet process extraction. They consist of starch granules that have not undergone any physical or chemical transformation. Depending on whether they are obtained from corn, wheat, potato or pea, native starches present different characteristics according to the size and structures of the starch granules, and depending on the ratio between amylose and amylopectin as summarized in Table 5.1. In particular, in our study we compared AMYLO, a high amylose (60%) maize starch, with respect to corn starch (CS) containing only 25 % of amylose.

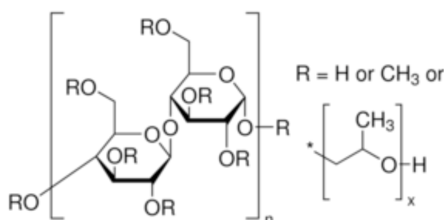
Table 5.1 - Amylose and Amylopectin contents (%) of native starches.

Components	Corn starch	Wheat starch	Potato starch	Pea starch	Amylo-maize starch N-400 (*)	Waxy maize starch
Amylose	25	25	20	35	60	1
Amylopectin	75	75	80	65	40	99

(*) High amylose starch.

5.1.2 HPMC

Hydroxypropyl methylcellulose (HPMC) is a semisynthetic, inert, viscoelastic polymer used as an excipient and controlled-delivery component in different medicaments to be administered for example orally, topically and ophthalmic; it is found in a variety of commercial products. It is a synthetic modification of the natural polymer, cellulose (Ghosal et al., 2011).



5.2 Preparation of FA-loaded Inserts

The inserts were prepared in the laboratory of Biopharmaceutics and Pharmaceutical Technology, Department of Pharmaceutical Sciences – University of Milan under the supervision of Prof. Gazzaniga.

The hot-melt extrusion technique was performed. First, AMYLO was granulated in a mortar with water (20% by weight with respect to the dry polymer) and glycerol (20% by weight with respect to the dry polymer); next, fluocinolone (3% by weight on the plasticized polymer) was added and the final mixture was extruded with no recycle through a twin-screw extruder (Haake MiniLab II, Thermo Scientific, USA) equipped with counter-rotating screws and a rectangular die (4x1 mm) at 100°C at 110 rpm. The resulting products were 1.0 mm thick, 4.0 mm wide and 250.0 mm long from which, through the use of a scalpel, inserts of appropriate size (1.0 x 4.0 x 12 mm) were obtained. The inserts, whose composition is shown in table 1, had a weight ranging between 0.033 g and 0.044 g.

5.3 Swelling and Dissolution Properties

Each insert was immersed in 4 mL of isotonic, 66.7 mM, pH 7.4 phosphate buffer solution (PBS) added of 20 μL of 0.02 %w/w methylene blue solution obtaining dye final concentration of 0.004 %w/w. The medium was kept at 37°C.

At predetermined time intervals, an image of each insert was captured using a digital microscope (Dino-lite Pro, ANMO, Taiwan). Computer analysis of the images was done using Dino Capture 2.0 Software (ANMO, Taiwan) to determine the ability of dye solution to penetrate into the inserts, transparency and shape retention of the inserts. End point of the test was loss of form, swelling, and/or the presence of fracture lines.

5.4 In *Vitro* FA Release Studies

At the beginning, in vitro release studies were carried out using Gummer-type vertical diffusion cells. Each insert (HPC-FA and AMYLO-FA), exactly weighed (FA average content: 1.3 mg), was introduced in a metallic tiny net support and placed in the receiving chamber of the diffusion cell that was hermetically sealed to avoiding the evaporation phenomenon. The receiving chamber contained 5 ml of PBS that was continuously replaced with fresh fluid at flow rate of 0.4 ml/min by a peristaltic pump (Minipuls 3; Gilson, Villers-le-Bel, France). The system was thermostated at 37°C. At predetermined time intervals, the fluid was withdrawn for analysis. A first part of the experiments lasted 52 h. Subsequently the release test was continued for 192 h only for AMYLO-FA insert. Both experiments were repeated 3 fold.

The release rate of FA from AMYLO-FA inserts was also determined using another experimental method by referring to Mruthyunjaya et al., 2006. Briefly, the inserts (FA average content: 1.3 mg) was put in contact with 1 mL of PBS in glass narrow vials maintained at 37 °C. The entire solution was withdrawn for analysis at 24 or 48-hours time intervals and replaced with fresh PBS. The tests lasted 25 days. In all cases the amount of released drug was determined by HPLC.

5.5 FA Analysis Method

The quantitative determination of FA in receiving phase was carried out by HPLC. The apparatus consisted of Shimadzu LC-20AT system with an UV-SPD-6AV detector and the injection valve was a Rheodyne with a capacity of 20 μL. A Kinetex Phenomenex® C18 100 A (5 μm; 150 x 4.6 mm) column was employed. The mobile phase consisted of a mixture of methanol: MilliQ-water (55:45). The detection wavelength was 238 nm, the flux was 1.0 mL/min, and the retention time was 8.35 min at the average pressure of 160 kgf/cm^2.

The amount of FA in the samples was determined by comparison with appropriate standard curve. It was determined with known FA-concentration solutions in the range of 0.004 μg/mL - 5.100 μg/mL. For the determination of the calibration curve the following procedure has been made: FA was solubilized in methanol and later progressive dilutions were made adding PBS (Chmielewska et al., 2006).

The amount of FA in the samples was determined by comparison with appropriate calibration curve that was linear (r^2= 0.983) in the range of 0.004 μg/mL - 5.10 μg/mL.

5.6 DSC Analysis

The possible drug-polymer interaction was studied by the DSC performed for fluocinolone acetonide (FA powder), pure polymer (AMYLO powder), physical mixture between drug and polymers (AMYLO-FA physical mixture in the ratio of 97:3; 70:30 and 50:50), unmedicated ocular inserts (AMYLO extruded), and medicated ocular inserts (AMYLO-FA extruded).

DSC analysis was performed using a Perkin Elmer differential scanning calorimeter (DSC 6, PerkinElmer, Italy). The apparatus was calibrated with purified zinc (99.9%). Samples (3–4 mg) were placed in flat-bottomed aluminum pan and heated at a constant rate of 20°C/min. Nitrogen, at the rate of 20 ml/min, was used as a purge gas during the role analysis. The specimens were heated from 30°C to 350°C.

The thermogravimetric curves were recorded with Pyris Instrument Managing Software, (Version 11, Perkin Elmer, Italy).

5.7 Preparation of VA-loaded Inserts

Inserts were prepared by employing freeze-drying technique. The matrices were prepared through the following procedure: first of all, HPMC was hydrated in MilliQ-water (1 %w/w) at room temperature for 12 h; 10 mg of VA was dissolved in 240 microliters of the polymeric dispersion to prepare five inserts. 50 μL of the final mixture were introduced in a silicon cylindrical plate with 3 mm diameter and were lyophilized using the following freeze drying process parameters:

- The *freezing* phase was carried out at - 40°C for 120 min, followed by an *extrafreeze* time at – 36°C for 10 min at a pressure of 400 Torr.
- The *primary drying* was carried out in four steps:

➢ Step 1: - 25°C for 360 min at 100 mTorr (rate: 1.8°C/h)
➢ Step 2: - 10°C for 240 min at 100 mTorr rate: 3.75°C/h)
➢ Step 3: + 5°C for 420 min at 100 mTorr (rate: 2.1°C/h)
➢ Step 4: + 25°C for 240 min at 100 mTorr

- The *secondary drying* was carried out at + 27°C for 120 min at 50 mTorr

Each freeze-dried insert contained 0.5 mg of HPMC and 2 mg of drug.

5.8 In *Vitro* VA Release Studies

In vitro release of VA from the lyophylized matrices (HPMC-VA) was investigated using Gummer-type diffusion cells with effective diffusional area of 1.477 cm^2. A previously hydrate cellulose ester membrane (Spectra/Por® 1 Dialysis Membrane, MWCO 6-8 kDa) was placed between the receiving and the donor compartments. 5 ml of PBS, kept at 32°C and stirred at 400 rpm, were used as receptor medium. The dialysis membrane was able to retain polymeric materials but to pass drug into the release medium. The matrices (donor phase) were put in contact with the membrane, moistened with 10 or 50 microliters of MilliQ water and the donor section was hermetically sealed. At predetermined time intervals, samples of the receiving phase were withdrawn for the analysis and replaced with an equal volume of fresh buffer. The amount of released VA was determined by HPLC. Each test was replicated at least 2 times.

5.9 VA Analysis Method

The quantitative determination of VA in receiving phase was carried out by HPLC. The apparatus consisted of a Shimadzu LC-20AD system with an UV SPD-10A detector equipped of autosampler SIL-10AD VP and a computer integrating system. A Kinetex Phenomenex® C18 100 A (5 μm; 150 x 4.6 mm) column was employed. The mobile phase consisted of a mixture of phosphate buffer: acetonitrile (90:10). The buffer solution consisted of monohydrated sodium dihydrogen phosphate (20 mM) and sodium hydrogen phosphate (30 mM) mixed in a ratio of 80:20. The detection wavelength, the flux and the retention time were 210 nm, 1.0 mL/min and 4.74 min, respectively, at the average pressure of 130 kgf/cm^2.

The amount of VA in the samples was determined by comparison with appropriate standard curve obtained by dissolving VA in MilliQ water and then diluting the resulting aqueous solution with PBS. The calibration curve was linear (r^2=0.998) over the concentration range studied (0.03504 μg/mL – 4.56 μg/mL).

6. Discussion and Results

The main goal of this thesis is the development of an ophthalmic biodegradable insert containing fluocinolone acetonide (FA), to apply in the posterior chamber of the eye. The inserts were prepared by research group of Pharmaceutical Technology of the Department of Pharmaceutical Sciences - University of Milan (Prof. Gazzaniga) by the hot-melt extrusion technique (HME). This technique is widely used to convert plastic raw materials into a product of uniform shape and density by heating and forcing them through a die, generally employing a rotating screw. It posses many advantages such as to overcome mixing and/or compaction problems with powder formulations, to eliminate the use of organic solvents. It is a viable technology to produce homogeneous drug incorporated polymeric matrices. Many researchers proposed HME for the preparation of drug delivery systems, fast dissolving or prolonged release formulations to be administered by oral, transdermal, transmucosal and transungual routes (Repka et al. 2007, 2012, Loreti et al. 2014, Melocchi et al. 2015).

The objective of the present investigation was to develop long acting ocular inserts to apply in the posterior segment of the eye.

Extrudates containing 3% FA and different polymeric materials (AMYLO, CS, CS-MS, HPC) were prepared, properly cut to give inserts of suitable size (1.0 x 4.0 x 12 mm) for ocular application and then undergone to a preliminary evaluation of swelling and dissolution properties. The inserts were put in contact with PBS, simulating biological fluids, and methylene blue dye solution was added to better follow the process. The dissolution process was observed for 5 hours. As shown in Figure 1a, during the first hour of contact, the surface of CS-FA and CS-MS-FA inserts appeared hydrate, swelled, partially broken but neither complete dissolution nor gel-like transformation had occurred. Conversely, AMYLO-FA surface appeared hydrate without swelling and dissolution. After 5 hours, CS-FA appeared too much swelled for its placement in the vitreous humour, because it might cause damage to the ocular structures. The same behaviour was observed for CS-MS-FA. For these reasons both of them were discarded. Based on these first observations, we decided to follow the behaviour of AMYLO-FA for further 12 days: during the entire period of observation no change had occurred in terms of dissolution and gel-like transformation over hydration and light swelling as well illustrated in Figure 1b.

Starch consists of two polysaccharides: amylose and amylopectin. Amylose is made up of α-(1→4) bond glucose molecules, it can exist in a disordered amorphous

conformation, and is not soluble in cold water whereas amylopectin is a highly branched polymer of glucose and water soluble.

Usually, normal starch contains 15-30% amylose, depending on the botanic origin, degree of maturity, and growing conditions of the plant. High-amylose starch contains 50% or more amylose like the AMYLO used in this study (60% amylose).

Amylose is able to reduce the crystallinity of amylopectin and how easily water can infiltrate the starch. Therefore, a high amount of amylose could decrease the degree of hydration. These characteristics might explain the different behaviour of CS and AMYLO.

Previously HPC-FA was selected as the best with respect to a wide range of devices tested; in this study AMYLO-FA appeared the best compared to inserts based on CS. Drug release performance of AMYLO-FA and HPC-FA inserts was comparatively evaluated. The two inserts were submitted to in *vitro* release study using the Gummer-type diffusion cell method (¶ 5.4), maintaining sink conditions (continuous receiving phase replacement), to evaluate the device influence on the FA release independently to the surrounding environment.

The release profile of AMYLO-FA and HPC-FA devices up to 52 hours is shown in Figure 2 and it indicates that the type of polymer greatly influenced the drug release. HPC-FA appeared to release quickly the drug: the main feature in this profile was an initial burst effect with approximately 10% of drug released in 2 hours followed by a slower release of remaining drug with approximately 89% of drug being released over the following 52 hours. The initial burst effect could be attributed to the portion of drug present in the surface of the insert, followed by the drug entrapped inside the insert. Related to drug release kinetics, the cumulative release data showed linearity with good coefficient of regression (r^2 = 0.9725) following Higuchi kinetics model. This model describes the drug release rate from a matrix, considering the geometry and the porous structure of the system. It can be expressed as:

$$M_t/M_\infty = K\sqrt{t}$$

where M_∞ is the absolute cumulative amount of drug released at infinite time (which would be equal to the absolute amount of drug incorporated within the system at time t=0), and K is a constant reflecting the design variables of the system. In general when applying Higuchi equation to controlled drug delivery system, it is important to consider that (a) the initial drug concentration in the system is much higher than the solubility of the drug; (b) the suspended drug is in a fine state such that the particles

are much smaller in diameter than the thickness of the system; (c) swelling or dissolution of the polymer carrier is negligible; (d) the diffusivity of the drug is constant and (e) perfect sink conditions are maintained (Siepmann and Peppas, 2001). These assumptions are not valid for most controlled drug delivery systems. However, due to the simplicity of the Higuchi equation, it is often used to analyse experimental drug release data to get a rough idea of the underlying release mechanism. Hence a proportionality between the cumulative amount of drug released and the square root of time is commonly regarded as an indicator for diffusion-controlled drug release. In our case, various phenomena (HPC swelling, polymer dissolution, concentration-dependent water and drug diffusion etc) seemed coexist and produced an apparent square root of time kinetics. Thus the mathematical model indicated that the drug release was mainly due to the diffusion and the polymer did not seem able to control FA release.

On the other hand, AMYLO-FA showed a remarkable reduction of FA release: in 2 hours only the 4% (2-3-fold lower than HPC-FA) of the total drug content was released followed by a slowdown (Figure 2) leading to a release of 36.13% of FA at 52 hours. Also in this case an initial burst effect was noted. Since in 52 hours only 36.13% of drug was released, the test was continued for 8 days and a slow but continuous release was obtained to reach 63.66% of FA released at the end of the experiment (Tab. 2).

Analysing the entire profile, it is possible to note that the release profile can be divided in two parts with different rate: in the initial hours (up to 6h), faster drug release happened with a rate of 15.65 μg/h (r^2=0.9560) followed by a decrease of release rate to 4.28 μg/h (r^2=0.9369) during the rest of time (up to 7 days). At the beginning there is the water uptake from the device with dissolution of drug on the surface; it has been previously demonstrated that AMYLO absorbs water up to 50% in 2 hours without change in volume. When the process reached the steady-state, a controlled drug delivery with zero order kinetics could be hypothesized.

The different rate on the hydration of inserts surely influenced the FA release: while HPC-FA appeared completely solubilized after 5 days, AMYLO-FA inserts maintained their shape without dissolution and gel-like transformation over hydration and slight swelling even after 12 days. This demonstrated that hydration, swelling and erosion/dissolution processes were only marginally influenced by manufacturing technique, and the dissolution rate of the drug in the microenviroment within the swollen matrix appeared to have a main role on driving drug release.

Afterwards, with this experiment, we can conclude that HPC-FA does not appear suitable to slowly release FA for a long time in the posterior segment of the eye, as

we set out to achieve. On the other hand, AMYLO-FA was considered interesting for further studies.

The release rate of FA from AMYLO-FA inserts was also determined using another experimental method by referring to Mruthyunjaya et al., 2006 (¶ 5.4). Two experimental protocols were performed, the first one replacing totally the receiving phase (t.r) every 24 hours, the other every 48 hours. FA release from the AMYLO-FA insert for both experiments was linear during the 25-day sampling period as illustrated in Figure 4. In similar experiments Jaffe et al (2000) and Mruthyunjaya et al. (2006) found the same trend. As summarized in Table 3, the release rates are 26.76 ± 0.44 and 10.61 ± 0.51 μg/day for a 24 h t.r. and 48 h t.r., respectively. At the end of the experiment (25 days), the total FA released were 674.10 ± 20.87 μg and 273.81 ± 10.08 μg for 24 h and 48 h t.r. respectively. All data are the average of two determinations ± standard error (SE).

The results showed that both the release rate and the amount of drug released at the end of the experiment decreased by half when the sampling at longer time (48 h) was carried out: so it seemed that the release of drug was mainly influenced and driven by the receiving environment (PBS) with respect to the device (insert). In this experiment, the change in the receiving phase was very slow to approach the biological conditions; in fact, the vitreous humor turnover is considered stagnant; the normal vitreous outflow is about of 0.1 μl/min (Park et al, 2005). In addition the thermal characteristics of AMYLO-FA insert were studied by using DSC analysis (¶ 5.6) to characterize the device from a physico-chemical point of view. The temperatures corresponding to the thermal transitions are summarized in Table 4 and graphically in figure 5 for single pure components (FA powder and AMYLO powder), physical mixture (AMYLO-FA physical mixture 97:3; 70:30 and 50:50), unmedicated ocular inserts (extruded AMYLO), and medicated ocular inserts (extruded AMYLO-FA). FA powder showed a thermal transition at 281.79°C corresponding to the melting point as reported in literature (Bartolomei et al., 1996). AMYLO powder had a thermal transition at 139.09°C. This transition temperature moved to 170.99 °C when AMYLO was subjected to hot-melt extrusion. The combination of AMYLO, FA and extrusion technique produced a single peak at 162.02°C; a shift of 20°C of the polymer peak was observed and FA peak completely disappeared in this thermogram. To verify the influence of the extrusion on the chemical-physical characteristics of polymer, DSC analysis of the physical mixtures was performed. AMYLO-FA physical mixture (97:3, the same ratio of the extruded AMYLO-FA) added of 20% glycerol, which is part of the composition of the insert extruded, had only a peak of 160.40°C. Even in this case

the characteristic transition of FA wasn't found; this might be due to the modest active drug content into the insert. For verifying this hypothesis we have done different ratio of polymer-drug physical mixtures. Both AMYLO-FA (50:50) and AMYLO-FA (70:30) blends showed the polymer peak at about 149°C, and the typical FA peak at about 282°C (Tab. 4). This means that our hypothesis might be true, and no alteration of the drug had occurred during the extrusion process.

The DSC analysis suggested that no polymer-drug interaction occurred, and this means that the release of the active compound might depend only on the polymer modified structure.

Further experiments will be performed to optimize the formulation of the insert by using in any case the hot-melt extrusion technique.

The second part of this thesis aimed at tuning up cylindrical matrices by freeze-drying for administration of vancomycin (VA) in the precorneal area to produce a sustained release of drug and consequently to reduce the number of applications. The matrices prepared were evaluated in term of drug release rate.

VA release profile from HPMC-VA was shown in Figure 6: blue line represents the results of the experiment where 10 μL MilliQ water was added to hydrate the matrix (donor phase) and pink line represents the results obtained hydrating the matrix with 50 μL MilliQ water. In the first experiment, the percentage of released VA was 8.73% of the total content of the drug, while in the second there was a 2-fold increase in drug release (20.29%). In any case HPMC-VA releases the drug very slowly, may be due to a drug-polymer interaction during the freeze-drying process. DSC analysis of the lyophilized matrix and the single components (VA and HPMC) would suggest this hypothesis. In fact freeze dried matrix produced only a peak of thermal transition at 147.81 °C while each component had a single peak one (VA) at 164.7 °C and the other (HPMC) 187.17 °C (Fig.7).

Further studies should be performed to improve the formulation.

Table

Table 1 - Composition of the inserts under study.

Insert	Polymer		Excipient		FA
	Molecule	**%w/w**	**Molecule**	**%w/w**	**%w/w**
AMYLO-FA	Amylo-maize starch N-400®	97	-	-	3
CS-FA	Corn starch	97	-	-	3
CS – MS-FA	Corn starch	92	Magnesium Stearate	5	3
HPC-FA	Hydroxypropyl cellulose	97	-	-	3

Table 2 - In *vitro* release parameters of FA from HPC-FA and AMYLO-FA using Gummer-type diffusion cells (continuous receiving phase replacement).

Insert	FA Released (%)	
	52 h	**190 h**
HPC - FA	89.01 ± 5.21	-
AMYLO - FA	36.13 ± 1.39	63.66 ± 4.18

Table 3 - In *vitro* release parameters of FA from AMYLO-FA using total receiving phase replacement (t.r.).

t.r.	Release Rate, μg/day	FA Release after 25 days, μg
24 h	26.76 ± 0.44	674.10 ± 20.87
48 h	10.61 ± 0.51	273.81 ± 10.08

Table 4 - DSC analysis data.

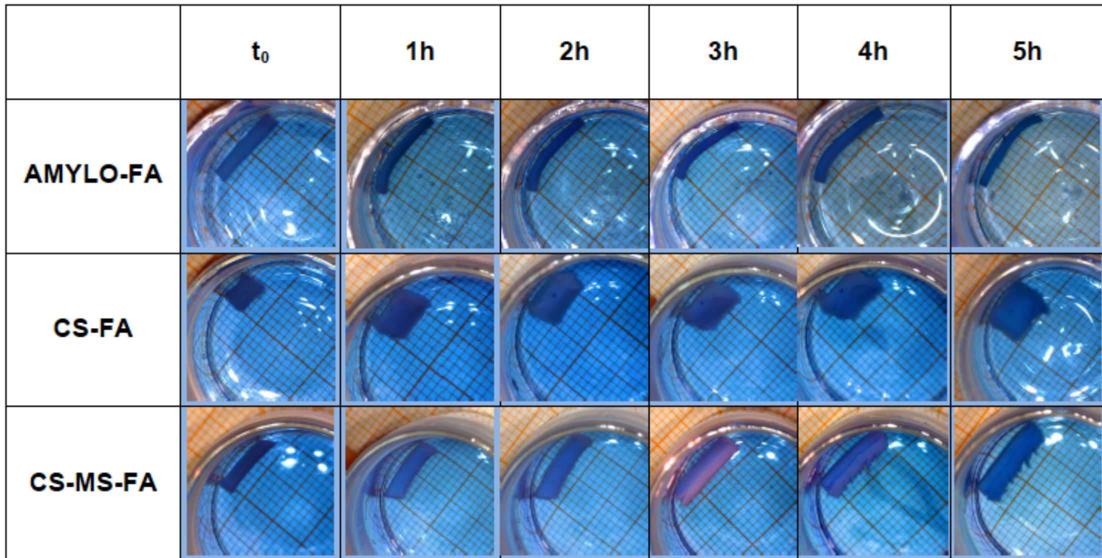
Component	Polymer Peak T (°C)	FA Peak T (°C)
FA powder	-	281.79
AMYLO powder	139.09	-
AMYLO-FA physical mixture (97:3)*	160.40	-
AMYLO-FA physical mixture (70:30)	149.64	282.87
AMYLO-FA physical mixture (50:50)	149.59	282.02
AMYLO Extruded	170.99	-
AMYLO-FA Extruded	162.02	-

*containing glycerol 20%.

Figure

Figure 1 - Swelling and dissolution properties of extruded inserts in PBS.
a) until 5 hours; b) until 12 days.

a)



b)

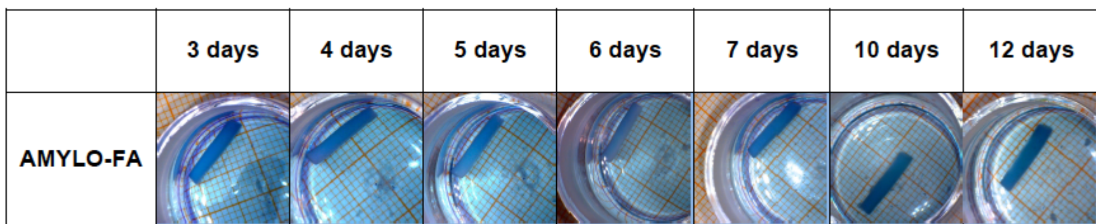


Figure 2 - FA release profile from HPC-FA and AMYLO-FA inserts up to 52h.

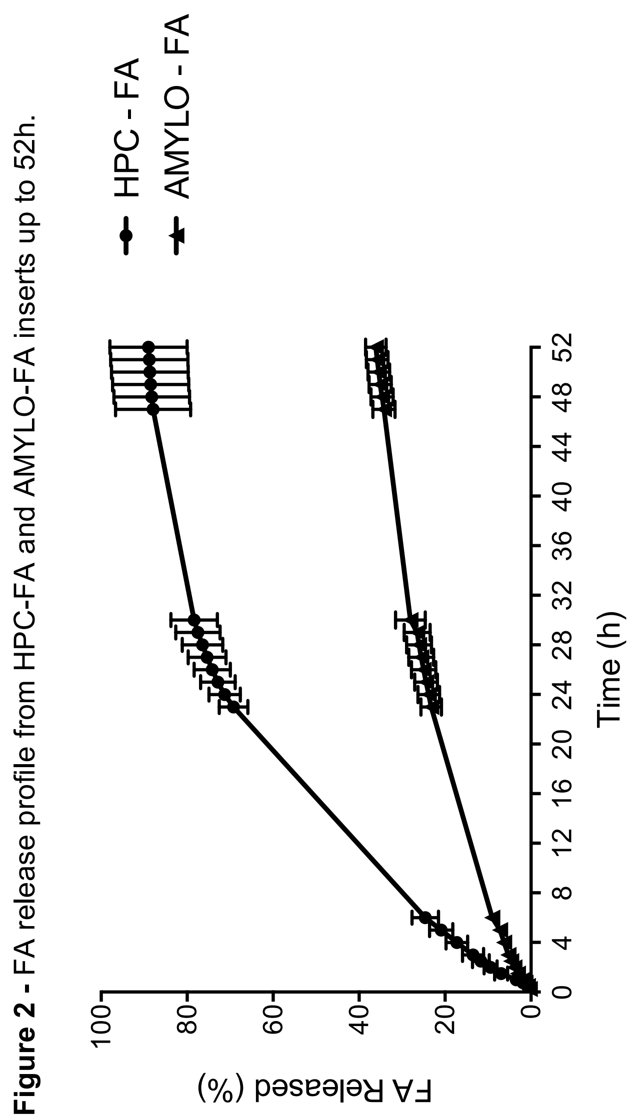


Figure 3 - FA release profile from AMYLO-FA insert up to 8 days.

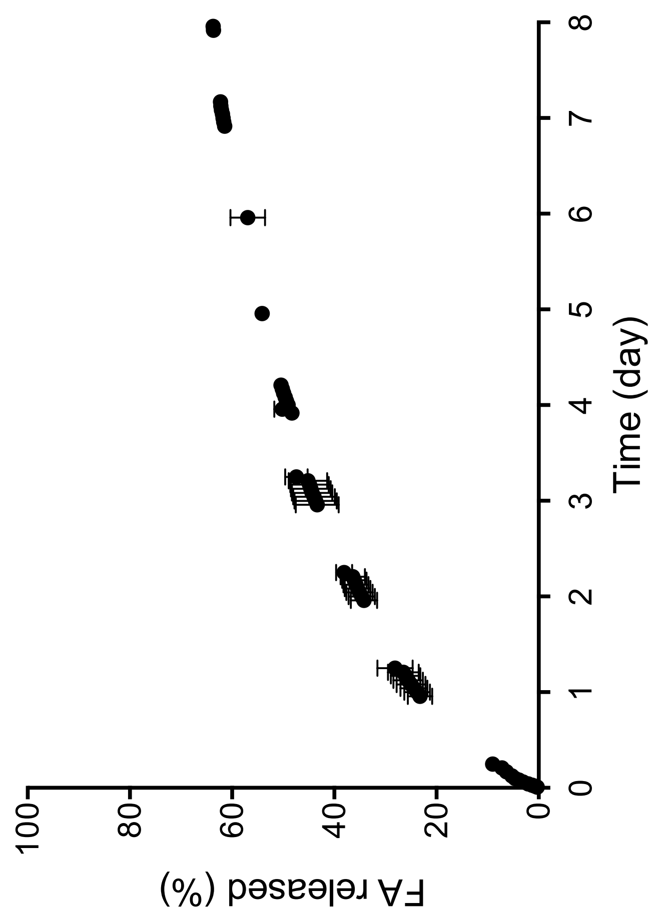


Figure 4 - Cumulative FA release (μg) from AMYLO-FA as a function of time. Total receiving phase replacement (t.r) every 24h.

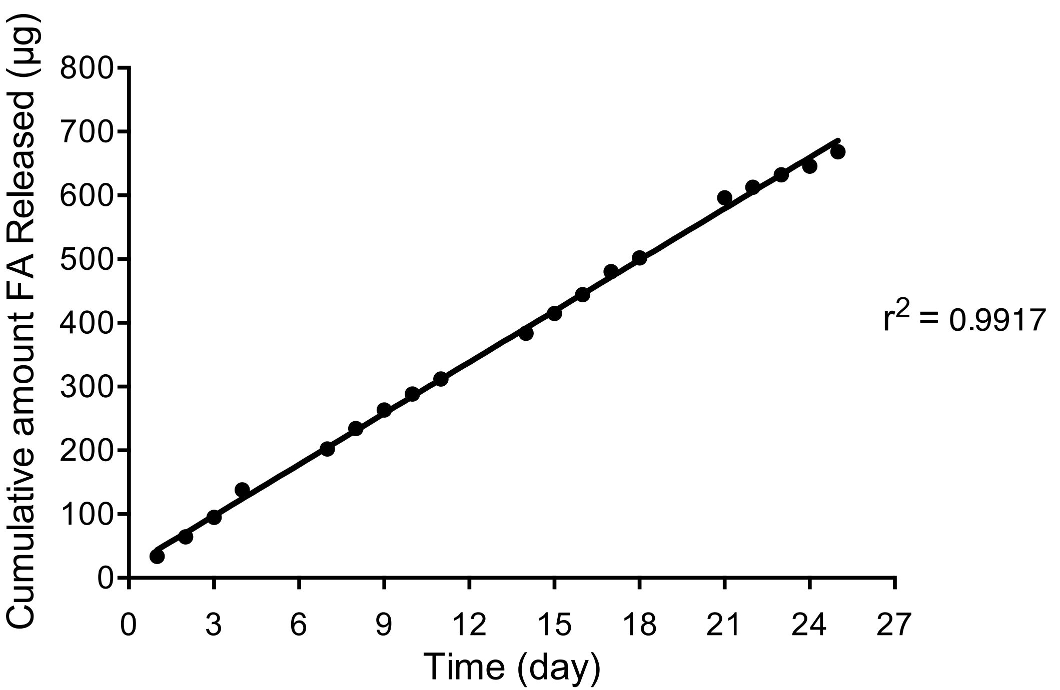
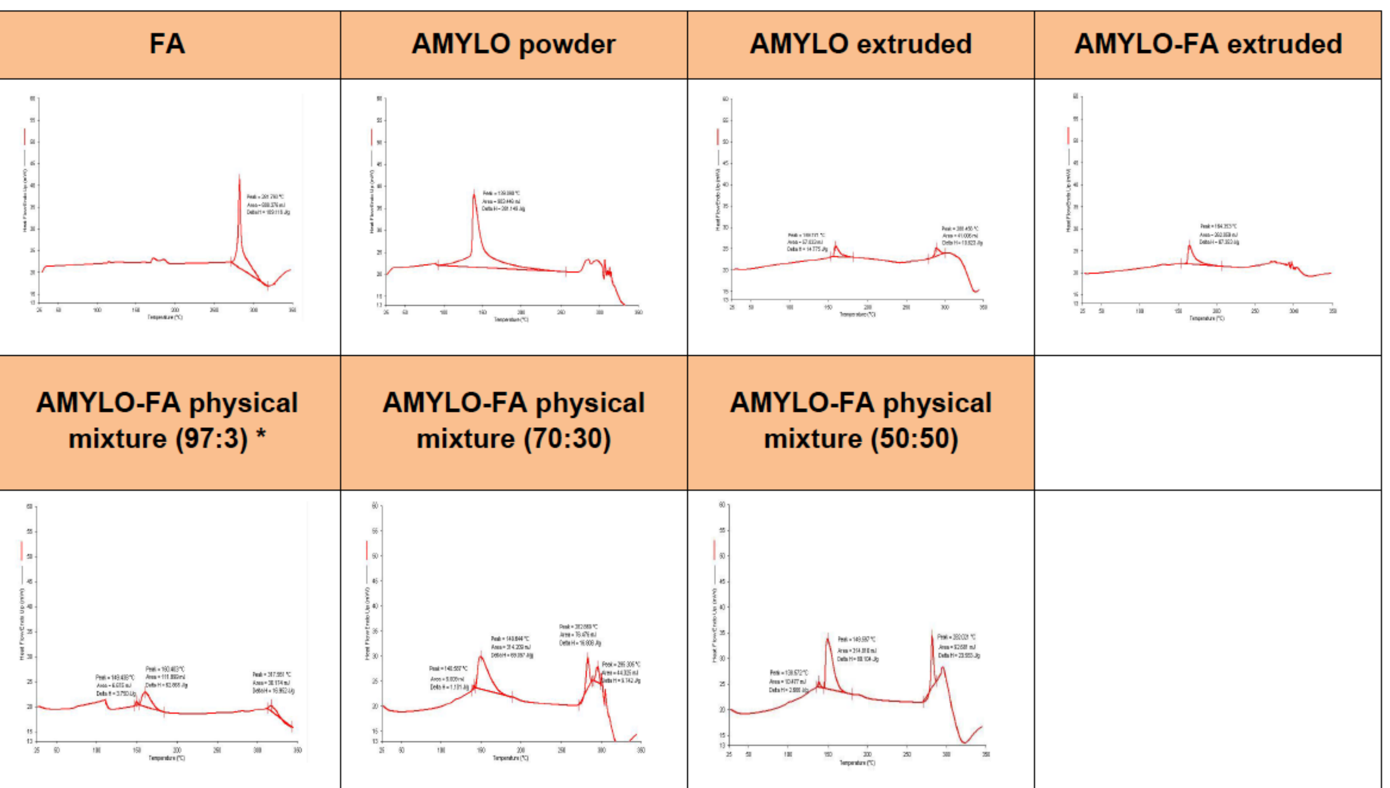


Figure 5 - DSC thermograms of the compounds under study.



* containing glycerol 20%

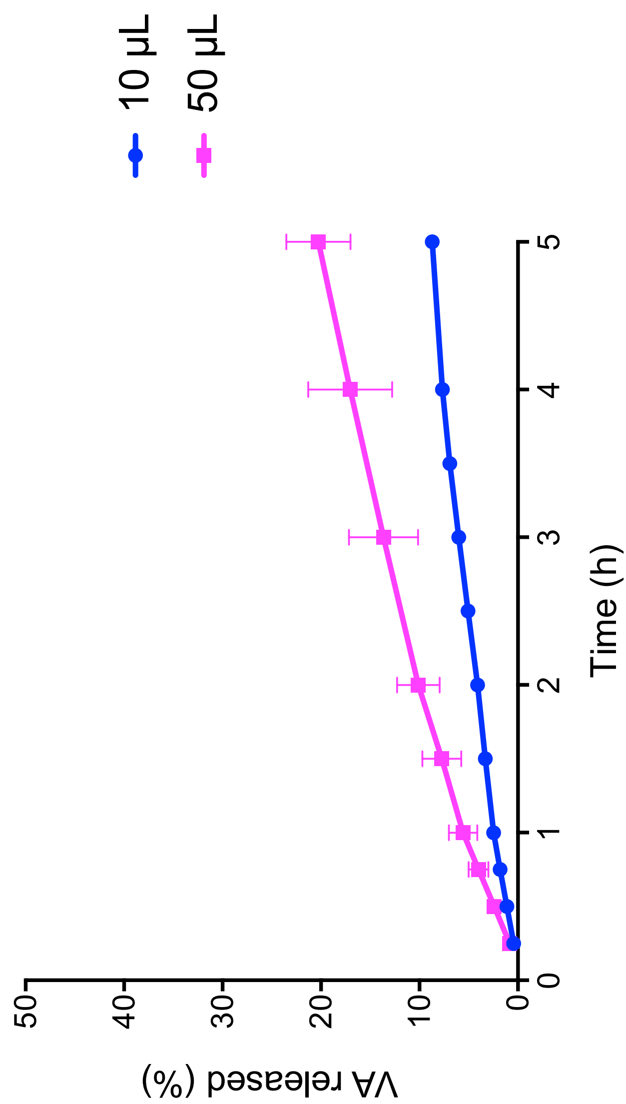


Figure 6 - VA release profile from HPMC-VA.

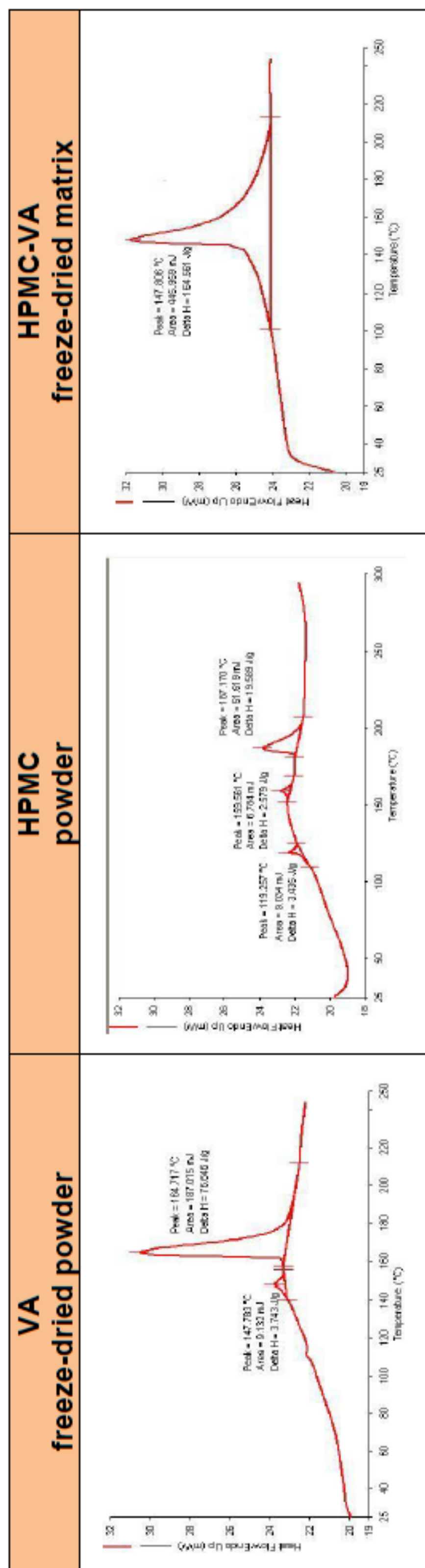


Figure 7 - DSC thermograms of the compounds under study.

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